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Identification of a Single Nucleotide Polymorphism in the *MxA* Gene Promoter (G/T at nt –88) Correlated with the Response of Hepatitis C Patients to Interferon

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Key Words
Hepatitis C virus · Hepatitis, chronic · Interferon · MxA protein · *MxA* gene · Single nucleotide polymorphism

Abstract
The interferon (IFN)-inducible MxA protein is known to play an important role in the host defense against certain viruses. We aimed to see if any genetic polymorphism in the promoter region of the *MxA* gene is associated with the IFN responsiveness of hepatitis C virus (HCV)-infected patients. Initially we sequenced the promoter region of the *MxA* gene in 12 subjects and found a polymorphic site. We then constructed a specific PCR-RFLP system for this site and subjected 63 samples from chronic hepatitis C patients who were nonresponders (NR) to IFN therapy to it, 52 with sustained response (SR), and 42 healthy controls. Subjects were all Japanese, and unrelated. A single nucleotide polymorphism (SNP) was identified in the *MxA* promoter region: G/T alleles at nt position –88. Interestingly, this SNP was involved in a genetic element highly homologous to the IFN-stimulated response element consensus sequence, and the G-to-T change there makes this homology a little greater. The rate of G·G homozygosity was 31% in the SR patients, significantly lower than in the NR patients (62%, $p = 0.0009$), while that of healthy controls was between the two groups (48%). Differences in HCV genotypes did not influence this result. Based on these findings, we propose that the SNP of the *MxA* promoter at nt –88 identified in this study affects the expression of MxA protein, and may thus be associated with the response of HCV patients to IFN.

Both viral and host factors may contribute to the phenomenon that some patients with chronic hepatitis C respond well to interferon (IFN) therapy but others do not: the rate of sustained response is lower than 50% [1]. Several factors related to the hepatitis C virus (HCV), such as low pretreatment load and/or genotype 2a, have been associated with better response [2, 3], but host factors have been only poorly understood. Previously we reported an association between the polymorphism of the mannose-binding lectin gene (*MBL*) and the IFN responsiveness of chronic hepatitis C patients in Japan [4]. We ascribed this association to the role mannose-binding lectin plays in the opsonisation of the HCV particles through the glycosylated coat proteins on the virion's surface.

It is possible that other host proteins with different functions may also be associated, through different mechanisms, with the IFN responsiveness of the HCV-infected patients. The MxA protein is one such candidate, because it is a protein that is induced by IFN and influences the IFN-induced antiviral activities of host cells against influenza viruses and several other viruses [5–7], although its antiviral effect against HCV per se has not been docu-

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mented. Recently, indeed, three reports suggested a role for the MxA protein in eliminating HCV in patients treated with IFN. Chieux et al. [8] reported that high levels of MxA protein were found in most of the IFN-treated hepatitis C patients in contrast to minimal levels before treatment; Fernández et al. [9] reported that the levels of the MxA protein were greater in virological responders than in nonresponders (NR), and Antonelli et al. [10] reported that the increase in the MxA mRNA from pretreatment levels to the levels at 8 weeks after the initiation of therapy was significant only in responders.

Why the MxA protein induction occurred in some patients but not in others remained obscure in these studies, however. We suspect that some genetic polymorphism of the *MxA* gene *(MxA)* may be involved. Here we report on the single nucleotide polymorphism (SNP) we identified in the promoter region of the *MxA* gene. The polymorphism at this SNP site is most likely associated with the levels of IFN-induced expression of the MxA protein, and thus further with the response of the hepatitis C patients to the IFN therapy.

One hundred and fifteen patients with histologically proven chronic hepatitis C who underwent IFN therapy and 42 anti-HCV-negative healthy individuals who visited our hospital for a health check were enrolled in this study. They were all Japanese, and unrelated to each other. Informed consent was obtained from every subject before collecting blood samples.

With respect to the responsiveness to IFN therapy, 52 were sustained responders (SR) in whom serum alanine amino transferase levels remained within the normal range and HCV RNA were continuously negative during the follow-up period of at least 6 months after the end of IFN therapy, while the other 63 patients were NR who remained positive for HCV RNA after IFN therapy irrespective of alanine amino transferase levels or who relapsed during follow-up. All of these patients had received IFN alpha and/or beta at a total dose greater than 300 million units. Pertinent to the HCV genotypes with which these patients were infected, the 1b genotype of the clade 1 was identified in 60, while the remaining 55 patients harbored the 2a or 2b genotype of the clade 2.

Nucleic acids, extracted from the peripheral blood mononuclear cells of patients and healthy controls, were subjected to PCR to amplify a 599-nt DNA fragment that covers the promoter region of *MxA*. Briefly, 0.05 μg of the nucleic acids were mixed with Taq-Gold (Perkin-Elmer) and the oligonucleotide primers #MXAF01 (5′-ACACACCCGTTTCCACCCTGGAGAGGCCAG-3′, forward, nt positions from −569 to −540) and #MXAR02

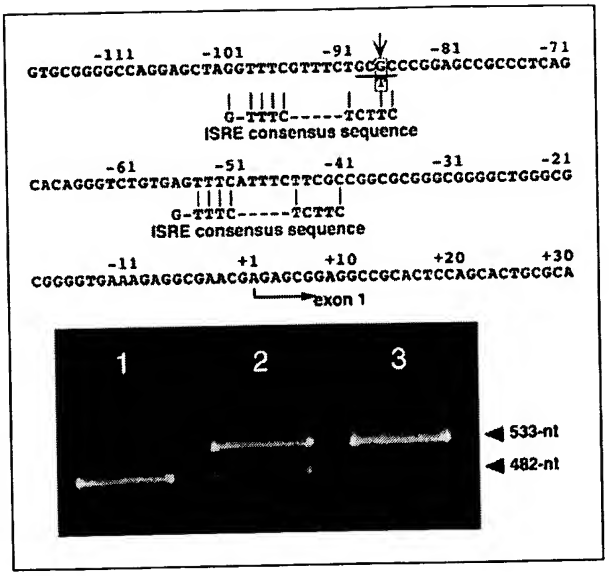

Fig. 1. The SNP in the promoter region of the *MxA* gene. The site of the polymorphism (G/T at nt −88) is indicated by an arrow, and the differential *Hha*I site is underlined. In the *Hha*I RFLP, lanes 1, 2, and 3 correspond to G·G homozygote, G·T heterozygote, and T·T homozygote, respectively (bottom).

(5′-TGCGCAGTGCTGGAGTGCGGCCTCCGCTCT-3′, reverse, nt +30 to +1; nucleotide position corresponds to that of HSMXAP, accession number X55639, reported by Chang et al. [11]), and reacted under the cycling conditions [95°, 10 min] + [95°, 10 s, 65°, 10 s, 72°, 60 s] × 55 + [72°, 7 min]. Twelve of the 157 samples were sequenced by directly reading the PCR products.

After identifying an SNP site within the amplified region by the sequencing study, we constructed an RFLP system for differential detection of the allelic nucleotides there. The 599-nt PCR products from all subjects were digested with *Hha*I (GCG↓C) and electrophoresed in agarose gels to see whether either or both of the 482-nt and 533-nt bands were generated.

Group data were compared using Fisher's exact probability test with or without Yates' adjustment. $p < 0.05$ was regarded as statistically significant.

A polymorphic site was identified in the promoter region of *MxA* by the initial sequencing study of 12 samples. It resided at nt position −88 (i.e., 88 nucleotides upstream from the exon 1) with single nucleotide alleles, G and T. This site was involved in a region which shows a considerable resemblance to the IFN-stimulated response element (ISRE) [12] as depicted in figure 1.

Table 1. Distributions of the *MxA* promoter genotypes among the 3 groups

MxA promoter polymorphism at nt −88	SR patients (n = 52)	NR patients (n = 63)	Healthy controls (n = 42)	p SR vs. NR
Allele frequency				
G	0.606	0.794	0.714	0.0018
T	0.394	0.206	0.286	0.0018
Zygosity				
G·G homozygote	16 (31)	39 (62)	20 (48)	0.0009
G·T heterozygote	31 (60)	22 (35)	20 (48)	0.0082
T·T homozygote	5 (10)	2 (3.2)	2 (4.8)	0.2956[a]

Figures in parentheses denote percentages.
[a] With Yates' adjustment.

Table 2. The G·G homozygosity is rare in SR patients irrespective of genotypes of HCV

Zygosity of the *MxA* promoter SNP at nt −88	SR patients	NR patients	p
In patients infected with HCV genotype 1b	18	42	
G·G homozygote	5 (28)	26 (62)	0.0321[a]
G·T heterozygote	12 (67)	14 (33)	0.0170
T·T homozygote	1 (5.6)	2 (4.8)	0.6051[a]
In patients infected with HCV genotype 2a or 2b	34	21	
G·G homozygote	11 (32)	13 (62)	0.0318
G·T heterozygote	19 (56)	8 (38)	0.1999
T·T homozygote	4 (12)	0	0.2722[a]

Figures in parentheses denote percentages.
[a] With Yates' adjustment.

In the subsequent *Hha*I RFLP study, the *MxA* genotypes with respect to the polymorphism at nt −88 were determined in all 157 samples. In this assay, samples with G at the polymorphic site showed a 482-nt band in the electrophoresis gel, while those with T showed a 533-nt band because the replacement of G by T there destroys the *Hha*I motif sequence (from GCGC to GCTC). In case of the heterozygote of G and T, both 482-nt and 533-nt bands showed up. As shown in table 1, the G·G homozygosity was significantly less frequently found in the SR group than in the NR group (31 vs. 62%, p = 0.0009), and the reverse was true for the G·T heterozygosity (60 vs. 35%, p = 0.0082). Even in allele frequency, SR patients had the G allele less frequently than NR with a statistical significance (p = 0.0018).

We divided the patients into two subgroups according to the genotypes of HCV with which they were infected (1b vs. 2a or 2b), and performed the comparison separately. As shown in table 2, the relative scarcity of the G·G homozygote in the SR patients compared to the NR patients was identified in both groups.

In the literature as well as in the DDBJ/EMBL/GenBank databases, the nucleotide at the −88 position of the human *MxA* gene has been described to be G, with the report of Ronni et al. [13] as an only exception: G was replaced with T in figure 1 of their paper [13]. In their paper, however, they did not describe any relevance of this substitution. We report here, therefore, for the first time the phenotypic relevance of the G/T polymorphism at this position.

Interestingly, this polymorphic site was involved in a genetic element having a high homology to ISRE consensus sequence [12], and the replacement of G by T at nt −88 makes this homology a little greater (fig. 1). There exists another ISRE-like sequence approximately 30 nt downstream from the G/T allelic site, and it has been reported that both of the ISRE-like sequences are important for the expression of *MxA* [12, 13]. Thus, we sus-

pected that the G/T polymorphism at the upstream ISRE-like sequence might affect the expression of *MxA*. Namely, we speculated that carriers of T might express the MxA protein more efficiently than those of G when given IFN.

A good model to test this hypothesis in clinical settings, before in vitro analyses, was derived from previous reports on the expression of *MxA* in patients with chronic hepatitis C treated with IFN [9, 10]. According to these reports, the IFN-induced increment in the levels of the *MxA* mRNA or protein was significantly greater in responders than in NR to treatment. Our present study seems to have corroborated these findings from a different point of view. That is, we found that the G·G homozygosity with respect to nt −88 of *MxA* was significantly rarer in SR patients than in NR patients (31 vs. 62%, p = 0.0009). The HCV genotype has been regarded as one of the most important factors to predict outcomes of IFN therapy: for example, HCV-1b of clade 1 is more resistant to IFN than HCV-2a/2b of clade 2 [3]. Our present results suggest that the *MxA* promoter SNP may function as another predictor, which is independent from at least the genotype of HCV (table 2). Most likely the IFN-induced expression of the MxA protein would occur less efficiently in the carriers of the G·G genotype than in those with the G·T or T·T genotype. If this is true, it is rational that the G·G genotype carriers, as compared to the G·T or T·T carriers, do not achieve the IFN-induced antiviral state which is enough to inhibit or eliminate HCV.

Our present findings and speculations should be corroborated by further studies including in vitro experiments, of course. Since we have sequenced only a very limited number of samples, it is possible that the nt −88 position has additional alleles, A and C, not found by us. Moreover, one or more other *MxA*-relevant polymorphism(s) may await discovery in the promoter region of *MxA* (in particular, in the downstream ISRE-like sequence) and/or in the coding region (exons and introns), some of which may affect expression and/or function of the MxA protein.

Apart from such basic aspects, however, our present findings are clinically very useful. The G/T polymorphism at nt −88 of the *MxA* gene can be used as one of the predictors of IFN responsiveness in patients with not only chronic hepatitis C but also other viral diseases for which type 1 IFN therapy is used. Ethnic differences in the *MxA* genotype distribution are also intriguing.

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